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THERMO-MECHANICAL AND BAKING PROPERTIES OF THE GLUTEN FREE ZEIN - STARCH DOUGHS

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Abstract
Thermo-mechanical properties of doughs based on zein and different types of starch (native corn starch, modified corn starch and tapioca) were investigated in this study. The physical properties of the gluten free breads, prepared using the model zein – starch mixtures, were additionally determined. The Mixolab parameters measured while running the Chopin+ protocol indicated that the dough exhibited viscoelastic properties at 39°C, and not at 30°C as in the case of gluten system dough. The type of starch used in admixture with zein to prepare the dough significantly influenced the viscoelastic properties of the samples. The lowest starch retrogradation was obtained for the zein-modified corn starch mixture (C5-C4 of 0.55 Nm), while in case of the samples with native corn starch and tapioca the C5-C4 values were close to 0.74-0.79 Nm. The zein-tapioca sample had the lowest breakdown (C3-C4) of 0.22 Nm, followed by the zein-native corn starch sample, with C5-C4 of 0.53 Nm. Bread samples prepared with zein and native or modified corn starch at high water absorption levels (77% and 85%, respectively) had the highest specific volume values of 3.18 cm^3/g and 2.22 cm^3/g, respectively. These results indicate that zein can be successfully combined with starch from various sources for obtaining gluten free products.

Keywords: zein, corn starch, tapioca, dough, thermo-mechanical properties, bread

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Introduction

The poor quality of the gluten free baked products is often due to the poor protein network and difficulties in supporting the formation of workable doughs (Smith *et al*., 2017).

Corn is a gluten free cereal that can be used as base flour in the gluten free baked products. Zein is the general name used for the alcohol soluble prolamines found in the endosperm of the corn, and accounts for ~60% of the total proteins content of the corn (Singh *et al.*, 2012). The poor water solubility of zein is due to the presence of high amounts of non-polar amino acids, such as leucine (19.3%), proline (9%), alanine (8.3%), phenylalanine (6.8%), and isoleucine (6.2%) (Singh *et al.*, 2012). Zein consists of four major fractions, namely α-, β-, γ- and δ-zein, having various solubility, and molecular weights, and particular amino acids sequence in the peptide chains (Kasaai, 2018; Bean *et al*., 2021). Alpha zein, which is highly hydrophobic and soluble in 70-95% aqueous alcohol solutions, represents over 80% of total zein (Zhang *et al.*, 2011; Kasaai, 2018). The commercial zein products are particularly rich in α-zein, with the secondary structure consisting primarily of α-helices (Lawton, 2002; Taylor *et al*., 2016; Bean *et al*., 2021).

Zein can be successfully used for producing films, coatings, fibers and different types of delivery systems for bioactive compounds, having therefore a wide range of application in medicine and fields like food and pharmaceutics industries (Bean *et al*., 2021; Giteru *et al*., 2021).

Despite the poor nutritional quality given by deficiency in tryptophan and lysine (Singh *et al*., 2012), zein was tested for developing different gluten-free products and received increased attention because of the ability to develop viscoelastic protein networks when kneaded above 25˚C (Lawton *et al.*, 1992). In addition to temperature, the energy input, mixing time and the overall composition of the gluten-free mixtures are important factors to be considered in the development of the zein dough (Bean *et al*., 2021). According Mejia *et al*. (2007), the ability of zein to develop protein networks is related to the structural rearrangements suffered during mixing at 35°C, resulting in the increase of the amount of amino acids organized as β-sheets in the detriment of α-helical structures, therefore favoring the formation of viscoelastic systems. Anyway, the β-sheet content gained over shear mixing, at temperatures above the glass transition temperature, is rapidly lost when shearing stops, because of the instable nature of the zein polymers (Mejia *et al*., 2007). Therefore, slight modification of zein structure is desired for improving its technological functionality in different matrices. Further study of Mejia *et al.* (2012) supported the hypothesis of improvement of the viscoelasticity of the zein-based dough by stabilizing the β-sheet structures though the addition of small amounts of casein or high molecular weight glutenin. The concept of zein-based "coproteins" appeared to be efficient for improving the stability of the viscoelastic fibrils, therefore supporting their use for obtaining gluten-free matrices with acceptable breadmaking properties (Mejia *et al.*, 2012). Other studies indicated the possibility of improving the β-sheet content of zein by the use of diluted organic acids (Sly *et al*., 2014) or hydrocolloids, such as hydroxypropyl methylcellulose

(Schober *et al*., 2007). In addition, Taylor *et al*. (2016) reported that the use of oxidizing agents, such as hydrogen peroxide and peroxidase, allows improvement of zein hydration and increasing dough extensibility. The confocal laser scanning microscopy images on the dough systems prepared with hydrogen peroxide showed that the starch granules are surrounded by continuous zein films, preventing clumping (Taylor *et al*., 2016). The increase of the zein β-sheet content and improvement of the elastic properties were as well reported by Federici *et al*. (2021) upon the thermal treatment at temperatures over 160°C. They showed that zein heating at 160-190 °C prior to mixing with 85% rice starch resulted in dough formulations with better extensibility when applying high extensional strain. Moreover, Federici *et al*. (2020) reported that extrusion process is capable to generate large zein aggregates, favoring the interactions with starch and ensuring the improvement of the elastic properties and strain-hardening behavior of the dough, which is important for the efficient retention of the gas during dough proofing.

The main ingredients (flours, starches, co-proteins, hydrocolloids) and the additives blended with zein, as well as the water used for preparing the dough might significantly influence the development of zein viscoelastic dough and its properties (Andersson *et al*., 2011; Federici *et al*., 2020; Bean *et al*., 2021). Andersson *et al*. (2011) noted that water level used to prepare the dough with zein have influence on mixing time and energy, while Federici *et al.* (2020) reported the influence of the type of starch blended with zein on mixing time.

The objectives of this study were to evaluate the thermo-mechanical properties of gluten-free mixtures obtained by zein blending with different types of starch and the physico-chemical properties of the bread samples prepared while considering two different water absorption capacity levels.

Materials and Methods

Materials

Zein (Z) (Sigma-Aldrich, Germany), corn starch (S) (SanoVita, Romania), modified corn starch (MS) (Clearam CR 30 20, Roquette, France) and tapioca (T) (SanoVita, Romania) were used in the experiment to prepare gluten-free doughs and breads.

The thermo-mechanical properties of composite flours

The model mixtures consisting of 10% zein and 90% starch were used for measuring the thermo-mechanical properties with the Mixolab device (Chopin Technology, Villeneuve La Garenne, France). Preliminary studies have been made in order to determine, for each zein – starch mixture, the water adsorption (WA) capacities suitable for determining the thermo-mechanical properties of the dough systems. For each investigated zein-starch mixture, one Mixolab test was performed at the WA level which ensured reaching the standard maximum consistency of 1.10±0.05 Nm when kneading the dough at 30°C, while a second test was performed at lower WA level, decided in such manner to allow appropriate dough system formation and to increase in the same time the glass transition temperature of the zein (Jeong *et al*., 2016). It was decided that for the Z+S mixture the investigations should be done at WA of 70 and 77%, for Z+MS at WA 77 and 85%, and for Z+T at WA of 85 and 98%. The Chopin+ protocol with 90 g dough weight was selected for running the thermo-mechanical tests, and the main torque values related to proteins behavior or starch performance within the dough matrix were registered as follows: maximum C1 torque recorded over the initial mixing stage at 30°C; CS – dough consistency measured after 8 min of mixing at 30°C; minimum C2 torque associated to proteins weakening while heating the dough; C3 providing information on the starch behavior at gelatinization, C4 associated to the stability of the gelatinized starch within the dough matrix, and C5 related to starch retrogradation while the cooling the dough to 50°C phase (Dubat and Boinot, 2012; Svec and Hruskova, 2015). Additional information on the starch behavior while heating and cooling the dough behavior were gathered by using the Mixolab torques values to calculate the mechanical weakening of proteins (MWP=(C1-CS)/C1×100), breakdown (C3-C4), starch retrogradation (C5-C4).

Breadmaking procedure

The zein-starch mixtures were further used for the breadmaking tests, according to the one stage procedure (Pătraşcu *et al*., 2017). The dough samples were prepared by well mixing in a bowl: 90 g starch, 10 g zein, 1.5 g salt, 1.5 g sugar, 3 g fresh baker's yeast (Rompak SRL, Pascani, Romania) and water according to the WA levels considered in the Mixolab measurements. Baking of the dough samples was further done at 180°C for 30 min, in an electric oven (Electrolux, Stockholm, Sweden).

Characterization of the breads

The bread samples were further analyzed in terms of texture properties, specific volume, and crumb color. All measurement were performed after cooling to samples at 22±1°C, within 24 hours after baking.

The specific volume of the zein-based breads was assessed using the rapeseed displacement method (SR 91:2007).

The ML-FTA (Guss, Strand, South Africa) instrument, fitted with a probe having diameter of 7.9 mm, was used to penetrate the bread slices by 25 mm at 5 mm/s speed and trigger threshold force of 20 g, for measuring the firmness of the samples. Three texture measurements were run on two different bread slices taken from each sample after removing the edges.

The Chroma Meter CR-410 (Konica Minolta Inc., Ramsey, NJ, USA) was used to measure the brightness (L*), presence of red shades (a*) and yellow shades (b*) on the crumb color.

Statistical analysis

All measurements were run in triplicate and results are reported as mean values together with standard deviation. The One-way ANOVA and post-hoc analysis using Tukey method at confidence of 95% were applied using the

Minitab19 (Minitab Inc., PA, USA) software, to find significant differences between the experimental results ($p < 0.05$).

Results and discussion

The thermo-mechanical properties of composite flours

Primary and secondary Mixolab parameters are shown in Table 1 and Table 2, respectively.

The thermo-mechanical properties of the zein-native corn starch mixture were investigated at WA of 70 and 77%. The zein-native corn starch dough prepared at WA of 70% exhibited, after 161 s of kneading at 30°C, the maximum consistency (C1) of 1.78 Nm, followed by consistency decreases to 1.62 Nm (CS) until the end of the 30°C step of the Chopin+ protocol. As the temperature begins to increase in the next Chopin+ step, the consistency of the gluten-containing dough systems decreases. Anyway, a different trend was noticed in case of the gluten free dough systems tested in the present study. Analyzing the results presented in Figure 1, one can see that the consistency of dough prepared with zein and corn starch followed an increasing trend at the beginning of the heating stage, defining a new torque peak of 2.02 Nm, after 630 s when reaching the temperature of 40°C. After this point, the dough consistency decreased to C2 of 0.53 Nm, registered after 1148 s at temperature of 76.4°C. Anyway, before reaching the C2 value, a rebound was observed at 1023 s and temperature of 67.4°C, the recorded C2' consistency being 0.68 Nm. The Mixolab curve of the zein-native corn starch dough prepared at WA of 77% had a similar trend, except for the second maximum torque recorded at 40°C, which was lower than C1 (Figure 1).

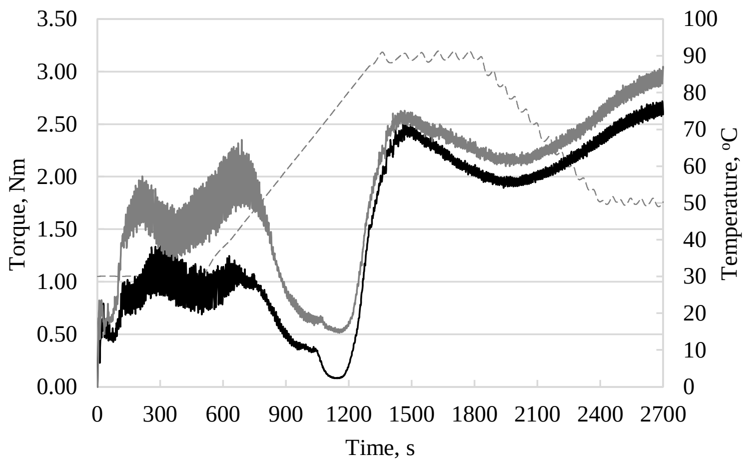

Figure 1. Mixolab curve of the zein – corn starch mixture at water absorption of 70% (gray) and 77% (black). The temperature regime during the Chopin+ protocol is shown with broken line.

According to Jeong *et al.* (2017), zein can form a viscoelastic network when kneading above its glass transition temperature. The same authors stated that the glass transition temperature of zein depends on the amount of water in the system. The higher water levels favor the decrease of the glass transition temperature (Jeong *et al.*, 2017), therefore explaining why the dough prepared at 77% WA showed viscoelastic behavior closer to that of a gluten-based one, compared to the sample prepared at 70% WA. The zein-based dough exhibited viscoelastic properties at temperature of 39°C, and not at 30°C as in the case of gluten-containing dough system. The mechanical weakening of proteins, calculated on the basis of C1 and CS, was 9.001% and 16.55% in zein-native starch dough with 77% WA and 70% WA, respectively. Jeong *et al*. (2017) studied the effect of zein levels in a composite flour with rice flour, and noted a twofold increase of the intensity of β-sheet structures in the dough with 10% zein at 40°C, compared to the dough prepared only with rice flour; the same WA was used for preparing both dough samples, decided such as to allow a maximum dough consistency of 1.10±0.05 Nm at 30°C. Jeong *et al.* (2017) suggested that these structural changes are responsible for the formation of viscoelastic dough in the case of zein-rice composite flour. Federici *et al*. (2020) suggested that for the same amount and type of protein, the type of starch used to obtain composites with zein has a significant effect on the viscoelastic properties of the gluten-free dough. The use of rice starch in admixture with zein resulted in dough having rheological properties comparable to the wheat-dough, whereas the use of maize or potato starch allowed obtaining dough samples with lower elasticity.

Table 1. Primary Mixolab parameters of the gluten-free composites prepared with zein (Z) and different types starches (corn starch, modified corn starch and tapioca) at different water adsorption capacities (WA).

Dough sample	**C1, Nm**	**CS, Nm**	**C2, Nm**	**C3, Nm**	**C4, Nm**	**C5, Nm**
Zein – Corn starch						
WA 70%	1.78±0.02[a]	1.62±0.01[a]	0.53±0.01[a]	2.54±0.01[a]	2.19±0.02[a]	2.97±0.01[a]
WA 77%	1.13±0.02[b]	0.94±0.01[b]	0.08±0.01[b]	2.44±0.01[b]	1.92±0.02[b]	2.66±0.01[b]
Zein – Modified corn starch						
WA 70%	2.87±0.02[a]	1.74±0.01[a]	1.62±0.02[a]	2.64±0.01[a]	1.23±0.01[a]	2.15±0.01[a]
WA 85%	1.05±0.01[b]	0.83±0.01[b]	0.55±0.01[b]	1.63±0.01[b]	0.84±0.01[b]	1.39±0.01[b]
Zein - Tapioca						
WA 85%	1.82±0.01[a]	1.46±0.01[a]	0.59±0.01[a]	1.19±0.01[a]	1.15±0.01[a]	2.73±0.01[a]
WA 98%	1.10±0.01[b]	0.95±0.01[b]	0.41±0.01[b]	0.90±0.02[b]	0.68±0.01[b]	1.46±0.01[b]

For an experimental set (the same type of starch), different superscript letters (a, b) on a column indicate significant differences at $p < 0.05$, based on Tukey test

Federici *et al*. (2020) explained the importance of the existence of small starch granules size in allowing the formation of a continuous protein network in the gluten free dough, which further influence to high extent the viscoelastic properties. Indeed, the size of the starch granules depends on the botanical origin, the rice starch having the lowest size distribution (3-8 µm), being followed by maize starch (1-20 µm) and finally by potato starch (15-110 µm) (Eliasson, 2004; Federici *et al*., 2020).

Analyzing the Mixolab curves in Figure 1, one can observe that the thermo-mechanical weakening of the zein within the gluten-free matrix occurs in two stages, regardless of the WA. In case of the dough prepared with WA of 70% the dough consistency first decreased to 0.68 Nm corresponding to the temperature of 67.4°C reached after 1023 s of mixing in the Mixolab bowl, followed by a new drop stage down to C2 of 0.53 Nm registered at 76.4°C. In a similar manner the consistency of the corn starch-zein dough prepared with WA of 77% decreased to 0.40 Nm (mixing time of 995 s and temperature of 65°C) in the first stage, followed by a new consistency decrease after additional 143 s mixing, when the C2 of 0.08 Nm was reached at 76°C (Figure 1). In both cases a narrow dough consistency plateau was observed at the end of the first stage of thermo-mechanical weakening at temperature of 65-67°C, when the corn starch gelatinization is initiated (Ubwa *et al*., 2012). These results indicate the advanced weakening of zein matrix during mixing and 76-77°C.

In the case of zein-native corn starch samples the maximum gelatinization, as indicated by the maximum C3 values, was achieved at temperatures of 89-90°C. The dough sample with WA of 70% presented lower breakdown (C3-C4) of 0.36 Nm, compared to samples with WA of 77%. Anyway, no significant differences ($p > 0.05$) between samples prepared with different water levels were observed in terms of starch retrogradation (C5-C4) (Table 2).

Table 2. Secondary Mixolab parameters (MWP - mechanical weakening of proteins; breakdown (C3-C4) and starch retrogradation (C5-C4)) of the gluten-free composites prepared with zein and different types starches (corn starch, modified corn starch and tapioca) at different water adsorption capacities (WA).

Dough sample	MWP, %	C3-C4, Nm	C5-C4, Nm
Zein – Corn starch			
WA 70%	9.00±1.05^{b}	0.36±0.03^{b}	0.79±0.03^{a}
WA 77%	16.55±1.57^{a}	0.52±0.02^{a}	0.74±0.02^{a}
Zein – Modified corn starch			
WA 70%	39.44±0.50^{a}	1.41±0.01^{a}	0.92±0.00^{a}
WA 85%	21.26±2.02^{b}	0.79±0.02^{b}	0.55±0.03^{b}
Zein – Tapioca			
WA 85%	19.45±0.22^{a}	0.04±0.00^{b}	1.58±0.00^{a}
WA 98%	13.07±1.31^{b}	0.22±0.00^{a}	0.78±0.01^{b}

For an experimental set (the same type of starch), different superscript letters (a, b) on a column indicate significant differences at $p< 0.05$, based on Tukey test

The thermo-mechanical behavior of the gluten-free dough samples prepared with zein-modified corn starch mixture is presented in Figure 2. The dough with WA of 85% appears more stable during kneading at 30°C, the consistency drop between C1 and CS being 0.22 Nm, significantly lower compared to the drop of 1.13 Nm registered in case of the samples with WA of 70% (Table 1). For both dough samples prepared with zein-modified corn starch composite, C2 is recorded at significantly lower temperature of 46-47°C, compared to the samples based on zein-native corn starch mixture, in which case the C2 were recorded at ~76°C. Unlike the dough prepared with native corn starch, a rebound (consistency increased to 0.73 Nm at 55°C after 848 s of mixing) was noticed in the modified corn starch-based samples with WA of 85%. After the rebound, the dough consistency slowly increased in the first stage over the next 120 s, followed by a rapid increase to C3. The dough prepared with zein-modified corn starch sample at 85% WA shows a maximum gelatinization of 1.63 Nm at 79°C, significantly lower compared to the dough samples prepared with WA of 70%. These results might be due to the lack of free water in the latter dough system, such as to ensure, in the first stage appropriate starch swelling and further optimal gelatinization. Breakdown and starch retrogradation values are significantly lower in case of the dough prepared at WA of 85%, compared to the sample with WA of 70% (Table 2).

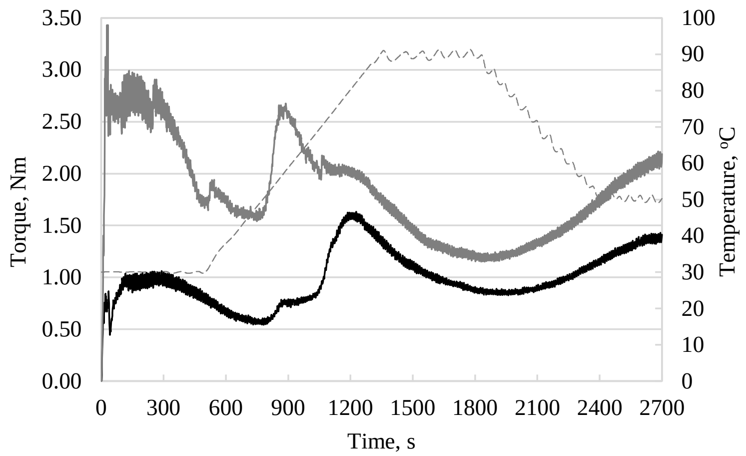

Figure 2. Mixolab curve of the zein – modified corn starch mixture at water absorption of 70% (gray) and 85% (black). The temperature regime during the Chopin+ protocol is shown with broken line.

Analyzing the thermo-mechanical behavior of the gluten-free dough samples prepared with zein-tapioca composite (Figure 3), one can observe that the sample with WA of 98%, which exhibited C1 of 1.1 Nm, presented higher stability at 30°C compared to the sample with WA of 85%. Thus, at the end of the initial kneading stage at constant temperature of 30°C, the CS measured on the sample with WA of

85% was 1.46 Nm, with 0.36 Nm lower than C1 (1.86 Nm), while the CS of the sample with WA of 98% was 0.95 Nm, lower by 0.15 Nm compared to C1. The temperature increase was accompanied by the dough consistency decrease to 0.71 Nm at 46°C, after 750 s of mixing in case of the sample prepared with zein-tapioca composite and WA of 98%, and to 1.26 Nm in case of the dough with WA of 85%. In case of both dough samples this decrease was followed by a rebound at 57-58°C (consistency of 1 and 1.59 Nm in case of the samples with WA of 98% and 85%, respectively), prior to consistency decrease to C2 of 0.59 Nm (for WA of 85%), and 0.41 Nm (for WA of 98%) registered at temperature of 80°C. Higher consistency values related to starch behavior (C3, C4 and C5) were recorded in the Mixolab curve of the dough with lower WA (Figure 3; Table 1). Anyway, the breakdown was lower in case of the sample with WA of 85% (C3-C4 of 0.04 Nm), compared to the samples with higher water level (C3-C4 of 0.22 Nm).

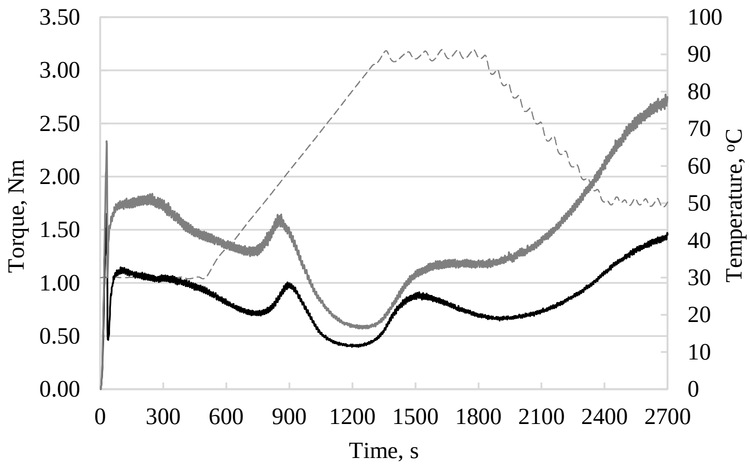

Figure 3. Mixolab curve of the zein – tapioca mixture at water absorption of 85% (gray) and 98% (black). The temperature regime during the Chopin+ protocol is shown with broken line.

Comparing the Mixolab curves of the three dough samples with different types of starches made at C1 of 1.1±0.05 Nm (native corn starch – WA of 77% in Figure 1, modified corn starch – WA of 85% in Figure 2, and tapioca – WA of 98% in Figure 3), one can see that the highest C3 value of 2.44 Nm was obtained in the case of native corn starch, followed by modified corn starch, 1.63 Nm, and tapioca 0.90 Nm. These values are recorded at different times, after 1400 s, 1200 s and 1500 s for native corn starch, modified corn starch and tapioca, but also at different temperatures – native corn starch and tapioca at 89°C, while modified corn starch at 79°C. In a study that focused on the thermo-mechanical behavior of the quinoa-based composite flour and different starch sources, Aprodu and Banu (2021) reported lower C3 values in samples with modified corn starch compared to native corn starch. The lowest starch retrogradation (0.55 Nm) was obtained for the zein-modified corn starch composite, while for the other two samples the (C5-C4) values were rather close (0.74-0.78 Nm). The zein-tapioca sample had the lowest breakdown, 0.22 Nm (98% WA), followed by the zein-native corn starch sample, 0.53 Nm (WA 77%). Aprodu and Banu (2021) reported much lower starch retrogradation values for samples with quinoa flour, dairy protein and modified corn starch compared to the native corn starch samples.

Bread characterization

The characteristics of the gluten free breads prepared with the zein-starch mixtures using different amount of water, in agreement with the WA used during Mixolab tests, are presented in Table 3.

Table 3. Physical properties of gluten free bread samples prepared with composites consisting of zein and different types starches (corn starch, modified corn starch and tapioca) at different water adsorption capacities (WA).

Bread sample	Specific volume, cm^3/g	Firmness, N	L*	a*	b*
Zein – Corn starch					
WA 70%	2.03 ± 0.06^{b}	7.28 ± 0.06^{a}	58.60 ± 0.25^{a}	2.08 ± 0.05^{a}	21.10 ± 0.12^{a}
WA 77%	3.18 ± 0.03^{a}	7.02 ± 0.07^{b}	57.98 ± 0.19^{b}	2.04 ± 0.04^{a}	20.88 ± 0.14^{a}
Zein – Modified corn starch					
WA 70%	1.75 ± 0.05^{b}	14.09 ± 0.08^{b}	59.02 ± 0.04^{a}	2.41 ± 0.01^{b}	27.25 ± 0.21^{a}
WA 85%	2.22 ± 0.07^{a}	26.86 ± 0.14^{a}	50.84 ± 0.10^{b}	4.40 ± 0.12^{a}	26.79 ± 0.51^{a}
Zein – Tapioca					
WA 85%	1.03 ± 0.05^{a}	10.59 ± 0.03^{a}	50.55 ± 0.05^{a}	3.74 ± 0.05^{b}	24.48 ± 0.29^{a}
WA 98%	1.10 ± 0.01^{a}	7.01 ± 0.06^{b}	47.09 ± 0.09^{b}	4.60 ± 0.27^{a}	24.09 ± 0.04^{a}

For an experimental set (the same type of starch), different superscript letters (a, b) on a column indicate significant differences at $p< 0.05$, based on Tukey test

Breads prepared with zein and native corn starch and modified corn starch at high WA levels had the highest specific volume values of 3.18 cm^3/g and 2.22 cm^3/g, respectively. These results might be explained by the better ability of the dough network to retain a larger amount of gas during baking (Elgeti *et al.*, 2014). Our observations regarding the higher specific volume of the bread samples based on the native corn starch compared to those based on modified starch are in agreement with Aprodu & Banu (2021). They reported that the partial substitution of quinoa flour with native corn starch resulted in bread with significantly higher specific volume in respect to the sample prepared with the same amount of modified corn starch. The WA level used to prepare the dough exerted a significant influence on the specific volume of the breads based on native and modified corn starch ($p < 0.05$). Aprodu *et al.* (2016) reported increasing volume of the gluten free breads prepared with increasing WA levels in the 55-105% range. They explained the importance of the existence of sufficient amounts of water in the system, such as to allow the appropriate hydration of all ingredients during kneading, and further on for supporting the suitable starch gelatinization during baking. The bread samples with zein and tapioca had the lowest specific volumes among all investigated samples, regardless of the WA level used (Table 3).

For all tested type of starch, the WA significantly influence the firmness of the bread samples ($p < 0.05$). A firmer texture was observed in case of the breads prepared with native corn starch or tapioca at lower WA levels, whereas in case of the bread prepared with modified corn starch, the firmness was higher for the higher WA level (Table 3). Anyway, it should be noted that the crumb of bread prepared with zein - modified starch mixture and WA of 85% was sticky, therefore influencing the accuracy of the firmness measurement. At low WA levels the starch might not have enough free water for optimal swelling and gelatinization, therefore resulting in baked products with high hardness (Nunes *et al*., 2009). Moreover, at low WA levels the intermolecular interactions between various ingredients of the gluten free systems are affected because of their competition for binding the available water, having a negative impact of the firmness of the bread (Hera et al., 2014; Aprodu *et al*., 2026). The lowest crumb firmness was registered in case of the bread with zein and native corn starch (7.28 and 7.02 N for WA of 70% and 77%, respectively), but a similar value was also obtained for the bread with zein and tapioca at 98% WA level (7.01 N).

The color properties of the bread samples were significantly influenced by the water addition level. The crumb of breads prepared with higher levels of water had lower values of the brightness (L*) (Table 3). Among all investigated samples, the breads with zein and tapioca had the lowest values of brightness and highest values of redness (a*). No significant influence of the water level addition on the yellowness values (b*) was noticed ($p > 0.05$). The lowest b* values were registered for bread with zein and native corn starch, and the highest values for bread with zein and tapioca (Table 3).

Conclusions

The type of starch used to obtain composites with zein influenced to high extent the thermo-mechanical properties of dough. The viscoelastic properties of zein have been manifested at 39°C during mixing, and not at 30°C as in the case of gluten system dough. Zein-native corn starch and zein-tapioca presented starch retrogradation values (C5) of 0.74 and 0.79 Nm, respectively, while in case of zein-modified corn starch the C5 was significantly lower. The lowest breakdown was registered in case of the tapioca-based dough, followed by the samples with native corn starch. The specific volume and crumb firmness of breads were influenced by the water level used to prepare the dough, but also by type of starch blended with zein. Better characteristics of breads were obtained when the higher amount of water was used. The highest specific volume was registered in case of sample with zein-native corn starch, and the lowest was obtained for sample with zein-tapioca.

The lowest crumb firmness was registered for breads with native corn starch or tapioca and high water levels.

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